A Convenient Synthesis of Cyclohexylammonium 2-Chlorophenyl 2-Cyanoethyl Phosphate

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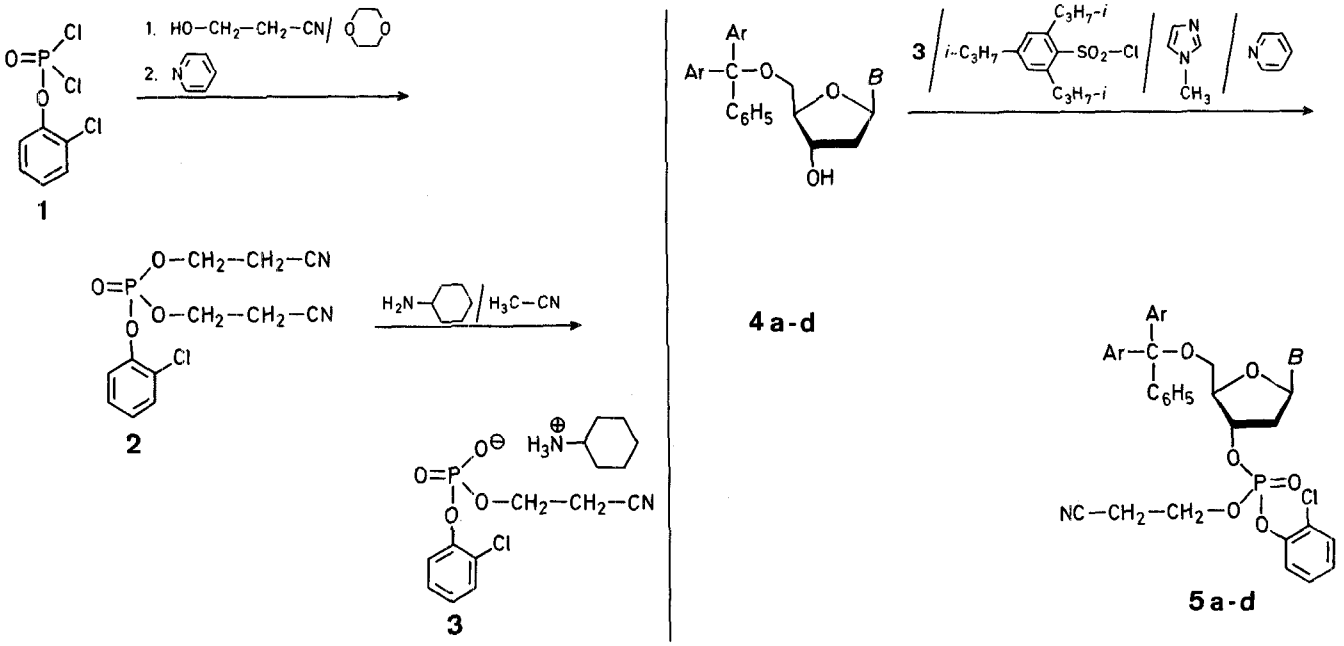
In the phosphotriester method of DNA synthesis, 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyribonucleoside-3′ 2-chlorophenyl 2-cyanoethyl phosphates[1,2] are important intermediates and a few phosphorylating agents for the synthesis of the fully protected monomers are known[3,4]. Recently, Vasseur et al.[5] have prepared a new and highly lipophilic phosphorylating agent, triethylammonium 2-chloro-4-tritylphenyl 2-cyanoethyl phosphate. On the other hand, Thuong et al.[6] have reported the synthesis of cyclohexylammonium 4-chlorophenyl 2-cyanoethyl phosphate by a four-step sequence and the application of the compound to the building blocks.

We describe here a practical and convenient synthesis of cyclohexylammonium 2-chlorophenyl 2-cyanoethyl phosphate (**3**). The reagent **3** is prepared from 2-chlorophenyl phosphorodichloridate (**1**) by a two-step sequence. Reaction of **1** with 2-cyanoethanol in the presence of pyridine gave quantitatively 2-chlorophenyl bis[2-cyanoethyl] phosphate (**2**), which was subsequently converted to **3** by treatment with cyclohexylamine.



According to the procedure of Efimov et al.[7], phosphorylation of 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyribonucleosides **4a–d** with **3** was carried out in good yield using a combination of 2,4,6-triisopropylbenzenesulfonyl chloride and 1-methylimidazole as a condensing agent. Our results demonstrate that the stable, crystalline compound **3** is a useful phosphorylating agent which is easily prepared from commercially available starting materials.

2-Chlorophenyl Bis[2-cyanoethyl] Phosphate (2):
To a solution of 2-chlorophenyl phosphorodichloridate (**1**; 11.4 g, 50 mmol) in dioxan (100 ml) is added 2-cyanoethanol (10.7 g, 150 mmol). Stirring is continued for 30 min and then pyridine (8.9 ml, 110 mmol) is added dropwise at room temperature. After

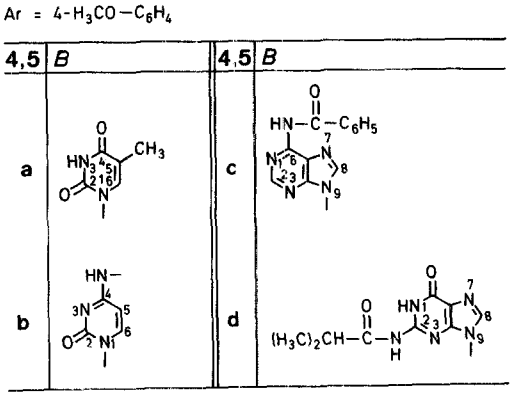


Table. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxyribonucleoside-3′ 2-Chlorophenyl 2-Cyanoethyl Phosphates **5a–d** prepared

Product	Yield [%]	T.L.C.[a,b] R_f value	Molecular formula[c]	^{1}H-N.M.R. ($CDCl_3$)[b,d] δ [ppm]
5a	83	0.53	$C_{40}H_{39}ClN_3O_{10}P$ (788.2)	1.40 (s, 3H, CH_3 of thymine); 2.75 (m, 4H, CH_2—CH_2—CN, H-2′, H-2″) 3.48 (m, 2H, H-5′, H-5″); 3.76 (s, 6H, OCH_3); 4.30 (m, 3H, CH_2—CH_2—CN, H-4′); 5.30 (m, 1H, H-3′); 6.42 (m, 1H, H-1′); 6.80 (d, J = 9.0 Hz, 4H_{arom}); 7.22 (m, 13H_{arom}); 7.52 (s, 1H, H-6 of thymine); 8.70 (br. s, 1H, NH)
5b	83	0.60	$C_{46}H_{42}ClN_4O_{10}P$ (877.3)	2.70 (m, 4H, CH_2—CH_2—CN, H-2′, H-2″); 3.48 (m, 2H, H-5′, H-5″); 3.76 (s, 6H, OCH_3); 4.34 (m, 3H, CH_2—CH_2—CN, H-4′); 5.30 (m, 1H, H-3′); 6.29 (m, 1H, H-1′); 6.80 (d, J = 9.0 Hz, 4H_{arom}); 7.22 (m, 16H_{arom}); 7.88 (dd, J = 7.7 Hz, 2.6 Hz, 2H_{arom}); 8.09 (dd, J = 7.7 Hz, 2.6 Hz, 1H, H-6)
5c	87	0.62	$C_{47}H_{42}ClN_6O_9P$ (901.3)	2.72 (dd, J = 5.1 Hz, 5.1 Hz, 2H, CH_2—CH_2—CN); 2.90 (m, 2H, H-2′, H-2″); 3.44 (m, 2H, H-5′, H-5″); 3.75 (s, 6H, OCH_3); 4.42 (m, 3H, CH_2—CH_2—CN, H-4′); 5.48 (m, 1H, H-3′); 6.52 (m, 1H, H-1′); 6.76 (d, J = 7.7 Hz, 4H_{arom}); 7.24 (m, 16H_{arom}); 8.04 (dd, J = 7.7 Hz, 2.6 Hz, 2H_{arom}); 8.17 (s, 1H, H-8); 8.68 (s, 1H, H-2)
5d	84	0.40	$C_{44}H_{44}ClN_6O_9P$ (883.3)	1.12 [m, 6H, $CH(CH_3)_2$]; 2.18 [m, 1H, $CH(CH_3)_2$]; 2.76 (m, 4H, CH_2—CH_2—CN, H-2′, H-2″); 3.36 (m, 2H, H-5′, H-5″); 3.76 (s, 6H, OCH_3); 4.35 (m, 3H, CH_2—CH_2—CN, H-4′); 5.55 (m, 1H, H-3′); 6.16 (m, 1H, H-1′); 6.78 (m, 4H_{arom}); 7.25 (m, 13H_{arom}); 7.75 (s, 1H, H-8)

[a] T.L.C. plates: Kieselgel 60 F-254 (Merck), solvent system: $CHCl_3/CH_3OH$ (10/1).
[b] Each product is identical on T.L.C. and by ^{1}H-N.M.R. with an authentic specimen prepared according to Lit.[2].
[c] Satisfactory microanalysis obtained: C ±0.40, H ±0.24, N ±0.32.
[d] 90 MHz-^{1}H-N.M.R. spectra.

4 h, pyridine hydrochloride is filtered off and the mixture is concentrated under reduced pressure. The oil obtained is partitioned between dichloromethane (200 ml) and 5% aqueous sodium hydrogen carbonate (200 ml). The organic layer is washed with water (200 ml), dried with anhydrous sodium sulfate, and concentrated to give compound **2**, which is used for the next step without further purification; yield: 15.4 g (98%); single spot by T.L.C. on silica gel in chloroform/methanol (10/1, v/v).

^{1}H-N.M.R. ($CDCl_3$/TMS, 90 MHz): δ = 2.87 (t, 4H, J = 7 Hz, CH_2CN); 4.43 (dt, 4H, J = 7 Hz, 7 Hz, $-OCH_2-$); 7.05–7.67 ppm (m, $4H_{arom}$).

Cyclohexylammonium 2-Chlorophenyl 2-Cyanoethyl Phosphate (3): Cyclohexylamine (2.2 ml, 19.6 mmol) is added to a solution of 2-chlorophenyl bis[2-cyanoethyl]phosphate (**2**; 3.1 g, 9.87 mmol) in acetonitrile (15 ml). The mixture is allowed to stand at room temperature for 1 h and cooled. The separated crystals are filtered off and washed with ether to give **3**; yield: 3.1 g, (87%). An analytical sample is recrystallized from acetonitrile; m.p. 114–116°C.

$C_{15}H_{22}ClN_2O_4P$	calc.	C 49.93	H 6.15	N 7.76
(360.8)	found	49.82	6.07	7.86

I.R. (KBr): ν = 2930 ($H_3N^{\oplus}-$), 2250 (CN), 1240 cm^{-1} (P=O).

^{1}H-N.M.R. ($CDCl_3$/TMS, 90 MHz): δ = 2.66 (t, 2H, J = 7 Hz, CH_2CN); 4.16 (dt, 2H, J = 7 Hz, 7 Hz, $POCH_2$); 6.93–7.58 (m, $4H_{arom}$); 8.21 ppm (br. s, 3H, $H_3N^{\oplus}-$).

^{31}P-N.M.R. ($CDCl_3/H_3PO_4$): δ = −7.79 ppm.

Phosphorylation of the Protected Deoxyribonucleoside 4a with Reagent 3; Typical Procedure:
A mixture of 5′-*O*-(4,4′-dimethoxytrityl)-thymidine (**4a**; 1.06 g, 1.95 mmol) and cyclohexylammonium 2-chlorophenyl 2-cyanoethyl phosphate (**3**; 1.41 g, 4 mmol) is dried by coevaporation with anhydrous pyridine three times and then dissolved in anhydrous pyridine (8 ml). 2,4,6-Triisopropylbenzenesulfonyl chloride (2.42 g, 8 mmol) and 1-methylimidazole (1.31 g, 16 mmol) are added to the solution. After 30 min, water (1 ml) is added to the mixture at 0°C and the solvent is removed under reduced pressure. The residue is partitioned between dichloromethane (50 ml) and 5% aqueous sodium hydrogen carbonate (50 ml). The aqueous layer is extracted with dichloromethane (2 × 50 ml). The combined organic layers are washed with water (50 ml), dried with anhydrous sodium sulfate, and concentrated to an oil. Trituration with *n*-pentane (300 ml) affords a solid, which is redissolved in dichloromethane and purified by flash column chromatography[8] on silica gel (Merck Kieselgel 60, 230–400 mesh; 50 g) in chloroform. Elution of the column with chloroform/methanol (50/1, v/v), followed by precipitation of the appropriate concentrated fractions from *n*-pentane, affords the fully protected monomer **5a**; yield: 1.61 g (83%).

Phosphorylation of **4b–d** with **3** is carried out similarly and yields **5b–d** (Table).

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